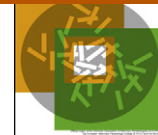




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## The production cost of anthelmintic resistance in lambs

C.M. Miller<sup>a</sup>, T.S. Waghorn<sup>a,\*</sup>, D.M. Leathwick<sup>a</sup>, P.M. Candy<sup>a</sup>, A-M.B. Oliver<sup>a</sup>, T.G. Watson<sup>b</sup>

<sup>a</sup> AgResearch, Grasslands Research Centre, Private Bag 11008, Palmerston North 4442, New Zealand

<sup>b</sup> Pfizer New Zealand Limited, Level 3, Pfizer House, 14 Normanby Road, Mt Eden, Auckland, New Zealand

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### ABSTRACT

The economic impact of anthelmintic resistance was investigated in lambs by comparing productivity parameters in groups of animals treated either with a highly effective anthelmintic, or an anthelmintic to which three species of resistant worms were known to be present. Ten farmlets, each stocked with 30 lambs, were rotationally grazed for 5 months, with monthly treatments of either albendazole, to which resistance existed, or a new combination product containing derquantel and abamectin (DQL-ABA) to which there was no resistance. Stock on five farmlets were treated with each anthelmintic and productivity measures, including liveweights, body condition and faecal soiling were assessed throughout. In addition, fleece weights and information on carcass weight and quality was collected at the end of the trial.

Anthelmintic efficacy was measured at the last two treatment dates by faecal egg count reduction test with larval cultures. Albendazole demonstrated efficacies of 48.4% and 40.9% for *Trichostrongylus* spp. and *Teladorsagia circumcincta* respectively. By contrast, the DQL-ABA treatments were >99% effective against all genera. The difference in live-weight gain was 9 kg in favour of the DQL-ABA treatments. This translated into a 4.7 kg increase in carcass weight with a 10.4% increase in carcass value. Significant differences in body condition scores, faecal breech soiling and fleece weights were also recorded, all in favour of the DQL-ABA treatments. The time required for 50% of the animals to reach a target live-weight of 38 kg was significantly shorter (by 17 days) in those animals treated with DQL-ABA.

The results show that the production cost of subclinical parasitism as a result of using an anthelmintic product which is less than fully effective due to resistance can greatly exceed the cost of routine testing of anthelmintic efficacy and the adoption of new anthelmintic classes. There is a strong case for many farmers to re-evaluate their position on some of these issues in order to optimise financial performance.

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### 1. Introduction

Gastrointestinal nematode parasites can have a significant impact on the productivity of grazing sheep (McLeod, 1995; Perry and Randolph, 1999) and parasitism is considered the most significant animal health issue by New Zealand sheep farmers (Lawrence et al., 2007).

While some farmers utilise strategies such as improved nutrition, changes in animal genetics and grazing management to minimise the impact of parasites, control is generally reliant on the administration of broad-spectrum anthelmintic drenches, particularly in regions where intensive agricultural systems are prevalent. Increasingly, the effectiveness of these anthelmintics is being threatened by the development of resistance to them in nematode populations.

Much research effort has been invested in understanding the factors which increase the development of

\* Corresponding author. Tel.: +64 6 3518086; fax: +64 6 3518134.  
E-mail address: [tania.waghorn@agresearch.co.nz](mailto:tania.waghorn@agresearch.co.nz) (T.S. Waghorn).

anthelmintic resistance and how these might be managed (Leathwick et al., 2009). However, adoption of resistance management practices on New Zealand farms has generally been poor, and this appears to be due to an element of confusion about what constitutes resistance management (Lawrence et al., 2007) and to a lack of a clear economic imperative to do so. Providing a general measure of the impact of anthelmintic resistance on animal productivity is difficult because there are invariably differences in farming systems, animal genetics and the size and pathogenicity of different nematode populations. A recent study conducted in New Zealand compared the productivity of lambs, under a monthly preventive program of anthelmintic treatments with either a new highly effective anthelmintic or one to which resistant parasites were present. The cost of anthelmintic resistance was in this case estimated at 14% of the value of the carcass at slaughter (Sutherland et al., 2010).

The aim of the current study was to extend our knowledge of the cost of anthelmintic resistance by comparing, under different conditions of pasture quality and quantity to the previous study, productivity in lambs treated with another highly effective anthelmintic compared to one to which resistance was known to be present. By accumulating sufficient evidence on the cost of using an anthelmintic product which is compromised by resistance it should be possible to convince farmers of the value of routine testing for anthelmintic efficacy, the first step in any resistance management programme, and of using products which are fully effective.

## 2. Materials and methods

A replicated field trial was run from March to September 2010 on the Flock House research farm near Bulls in the Manawatu region of the North Island of New Zealand. Ten farmlets, each of eight paddocks, were established and rotationally grazed with lambs for the duration of the study. All animals received a series of five treatments, at 28-day intervals, with either a newly introduced combination anthelmintic product (DQL–ABA, STARTECT®, Pfizer New Zealand Ltd, Auckland, New Zealand) or with albendazole (Albendazole, Merial Ancare New Zealand Limited, Auckland, New Zealand). The experimental design was therefore two treatments with five replicates in a completely randomised structure.

### 2.1. Site preparation

The site had been used in a previous study and was known to be contaminated with benzimidazole-resistant *Teladorsagia* (= *Ostertagia*) *circumcincta*, *Trichostrongylus colubriformis* and *Nematodirus spathiger*. To avoid any possible confounding of resistance levels as a result of the previous study, paddocks were allocated to farmlets in a restricted randomisation that ensured a balanced representation of the previous treatment structure in all farmlets i.e. two paddocks from each of the previous four treatments were allocated to every farmlet.

### 2.2. Animals

Three hundred castrated male lambs were purchased from external sources and transported to the research farm. On arrival, all were treated orally with ivermectin (Ivomec Liquid for Sheep and Goats®, Merial Ancare NZ Ltd) and an albendazole + levamisole combination product (Arrest Hi-Mineral®, Merial Ancare NZ Ltd) on consecutive days, at the manufacturer's recommended dose rates. Samples taken for faecal nematode egg count (FEC) seven days post-treatment confirmed that these treatments had effectively removed existing parasite burdens.

Lambs were then allocated to 10 groups of 30 based on liveweight, before each of these groups was allocated to a farmlet. All 30 animals were rotationally grazed on each farmlet throughout the trial, with a shift approximately every five to seven days. Lambs allocated to Treatment 1 were administered DQL–ABA, while Treatment 2 lambs were given albendazole. Both products were administered at the manufacturer's recommended dose rate of 2.0, 0.2 and 4.75 mg/kg for DQL, ABA and albendazole, respectively. All doses were calculated based on the heaviest lamb in each mob on the day of treatment.

Experimental manipulation of animals was approved by the Grasslands Animal Ethics Committee, Palmerston North, New Zealand (#12045). Under this approval any animal which had a FEC >3000 epg, or which lost >10% body weight between weighing (28 days), was treated with DQL–ABA and removed from subsequent analysis. Over the duration of the trial this occurred in nine animals, all of which remained on the site in order to keep animal numbers constant across farmlets.

### 2.3. Parasitology

Nematode populations in all the lambs were monitored using FEC, conducted at 4-weekly intervals, which usually coincided with the day lambs received their anthelmintic treatments. The number of strongylid and *Nematodirus* spp. eggs present was determined using a modified McMaster method in which each egg counted represented 50 epg (Lyndal-Murphy, 1993).

Seven to 10 days after each treatment, additional samples were collected for FEC in order to estimate the efficacy of the treatment. For the first three drench dates only 10 animals from each farmlet were sampled, however, for the last two dates all lambs were sampled. Efficacy was estimated by comparing the pre- and post-treatment mean FEC for each group of lambs, and calculating the percentage reduction in undifferentiated FEC.

To monitor the generic composition of eggs passed by the lambs, faecal larval cultures were carried out after each monthly faecal sampling as well as at the 4th and 5th post-treatment samplings. Faecal material surplus to that required for FEC was bulked within each farmlet and incubated at 24°C for at least 14 days. Infective third-stage larvae (L3) were extracted by baermannisation (Hendrix, 1998), concentrated in a water column by sedimentation at 5°C, and the generic composition of the first 100 L3 recorded.

Samples to estimate L3 numbers on pasture were collected at the beginning and end of the experiment from 5 of the 8 paddocks in each farmlet. Pasture was collected by hand-‘plucking’ herbage samples while walking zigzag transects across each paddock, until 200–300 g had been collected. Larvae were extracted from the samples by baermannisation, identified to genus and counted.

#### 2.4. Productivity measures

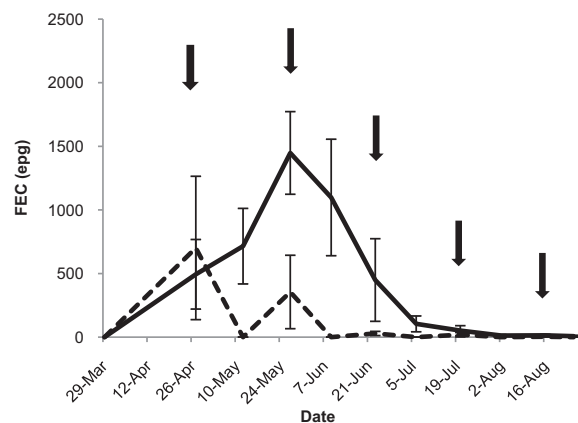
Liveweights were recorded monthly for all animals. Faecal soiling around the breech (dags) was assessed visually and given a score between 0 and 5; a 0 indicating no soiling and a 5 indicating severe soiling of the breech and rear legs. Body condition score was also assessed, on a 1–5 scale, by manual palpation of the lower vertebrae as an indication of the amount of fat covering the pelvic vertebrae (Shands et al., 2009), with a score of 1 indicating very poor condition and a score of 5 indicating overly fat. These parameters were assessed on three occasions during the trial, at the time of the 1st, 3rd and 5th anthelmintic treatments. At the end of the trial, lambs were shorn and fleece weights individually recorded. Animals were subsequently slaughtered at a commercial abattoir, and carcass weights and standard measures of quality recorded for analysis.

#### 2.5. Statistical analysis

The experimental unit in this experiment was the group of lambs grazing each farmlet and its associated nematode population. The design therefore involved two treatments with five replicates in a completely randomised structure, yielding an ANOVA table with 1 degree of freedom for treatment and 8 degrees of freedom for error. The data for analysis was the mean value for each farmlet.

Liveweight gain, body condition score, dag score, the number of L3 on pasture and the generic composition of pasture plucks were all analysed by ANOVA (proc GLM) in Minitab (Minitab 15.1, Minitab Inc 2006, USA) using a statistical model which included treatment anthelmintic (DQL-ABA or albendazole). For lamb liveweight gains, initial weight was included as a covariate in the model and for fleece weights the liveweight prior to shearing was used as a covariate. FEC at the time of each anthelmintic treatment was compared by calculating a geometric mean (GM) FEC for each farmlet, after transformation of the individual egg counts by  $\ln(x+1)$ , and testing for a difference in GM FEC between treatments by ANOVA as above. Post-treatment FECs were not compared statistically because the number of animals sampled was often low and the groups treated with DQL-ABA nearly always returned zero egg counts, and therefore zero variance. Post-treatment FECs are however included in Fig. 1.

The proportion of different genera in the faecal cultures was also analysed by ANOVA (proc GLM) but with a model which included farmlet, time and treatment. Repeated measures analysis was not used because there was an anthelmintic treatment between each time event (i.e. sampling) which would have eliminated, or partially eliminated, the worm burden after each measurement, effectively making the culture results independent.



**Fig. 1.** Treatment mean faecal nematode egg counts ( $\pm 95\%$  CI) from mobs of lambs treated five times with DQL-ABA ( $n=5$ ) (—) or albendazole ( $n=5$ ) (---) over the period of the experiment. Arrows indicate the anthelmintic treatment points.

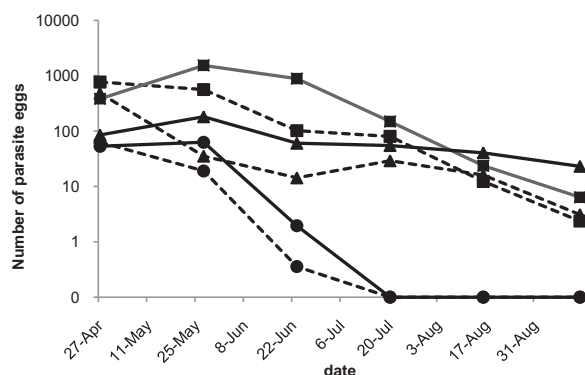
Time to target weight data was analysed in GenStat (GenStat v12, VSN International Ltd, UK) using repeated measures analysis of variance with the least significant difference (LSD) reported at the 5% level. Logistic curves were fitted to the data with day from start as the explanatory variable, and the time taken for 50% and 90% of the animals in each treatment group to reach the target weight was estimated using proc GLM with a binomial distribution and probit link function.

### 3. Results

GM FEC did not differ between the two treatments at the start of the experiment ( $P=0.386$ , Fig. 1). However, at all subsequent treatments the albendazole treated groups had GM FECs which were significantly higher, or which approached significance, than the DQL-ABA treated groups ( $P<0.001$ ,  $P=0.007$ ,  $P=0.061$  and  $P=0.005$  for treatment times 2–5 respectively). These differences undoubtedly reflected the different efficacy of the two treatments, with FEC in the albendazole-treated lambs remaining elevated after each treatment while that of the DQL-ABA-treated animals dropped to zero or near zero (Fig. 1).

The proportion of different genera present in the faecal cultures did not differ between treatments ( $P>0.30$ ). However, overall the proportions did change over time ( $P<0.001$ ). *Trichostrongylus* was the dominant genus at the start of the trial, making up 60% of the L3 present, whereas *T. circumcincta* made up 90% of L3 recovered at the end. To clarify how populations changed over time, FECs were apportioned by the percentage of each genus found in the matching faecal cultures to generate a FEC for each genus over the duration of the trial (Fig. 2).

The efficacy of the two anthelmintic products, based on FEC reduction, was markedly different (Fig. 1). DQL-ABA had an average efficacy over the whole trial of 99.9% and 100% while, albendazole had efficacies of 30% and 72%, against strongyles and *Nematodirus* spp., respectively. Following the last two treatments, faecal cultures from all the treated animals allowed the efficacies against different



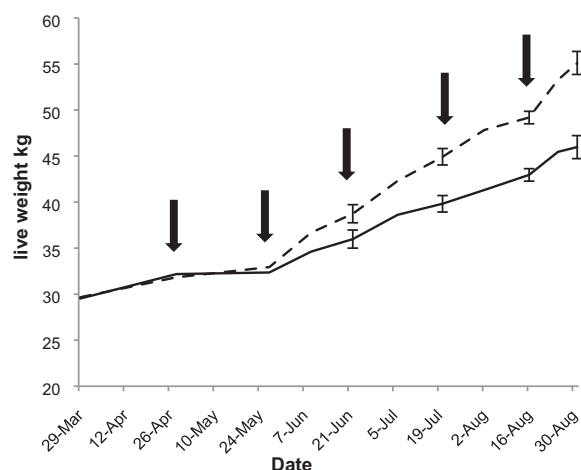
**Fig. 2.** Number of nematode eggs of each genus (*Trichostrongylus* – ■, *Teladorsagia* – ▲, and *Nematodirus* – ●) present in the pre drench cultures for the two treatments (— (DQL-ABA) and --- (albendazole)) over the period of the experiment. Total FEC was allocated to different genera based on the proportion of L3 recovered from faecal cultures.

strongyle genera to be determined. The average efficacy, over the last two treatments, against *Trichostrongylus* spp. was 99.7% and 48.4% for DQL-ABA and albendazole, respectively; while for *T. circumcincta* efficacies were 99.9% and 46.2%, respectively.

There was no difference between treatments in the condition or faecal soiling scores at the start of the trial (condition scores, 2.19 vs 2.26 and faecal soiling scores 0.32 vs 0.10 for the DQL-ABA and Albendazole treatments respectively,  $P > 0.2$ ). However, at the third and fifth treatment dates the DQL-ABA-treated lambs had higher condition scores and lower faecal soiling scores than the albendazole-treated lambs (Table 1).

Fleece weights at the end of the trial were significantly heavier in the DQL-ABA groups ( $P < 0.01$ ), producing 360 g more wool per animal, than the albendazole-treated animals.

Over the duration of the trial the animals treated with DQL-ABA gained an average of 9 kg liveweight more than those treated with albendazole ( $P < 0.001$ , Fig. 3). This difference in liveweight translated into a difference in carcass weights at slaughter of 4.7 kg heavier ( $P < 0.001$ ) for the DQL-ABA-treated animals, with a mean fat depth



**Fig. 3.** Mean liveweights ( $\pm 95\%$  CI) of lambs treated with either DQL-ABA (—) or albendazole (---) over the period of the trial. Anthelmintic treatment points are indicated by arrows.

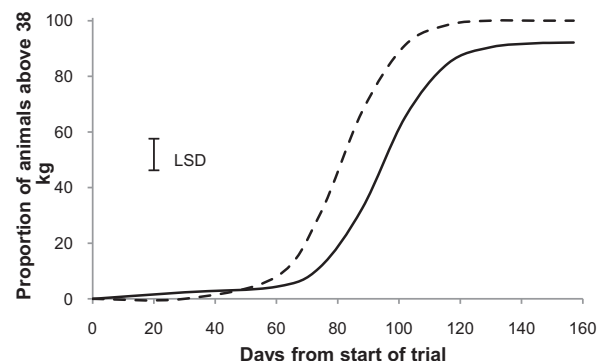
3.6 mm ( $P < 0.001$ ) greater, than that on the albendazole animals. The overall price gain for the DQL-ABA animals was NZ\$11.56 per animal.

The time taken by animals in the two treatments to reach a target weight of 38 kg differed significantly ( $P = 0.01$ ; Fig. 4). The time required for 50% of the animals to reach 38 kg was 17 days ( $\pm 0.5$ ) shorter in the DQL-ABA-treatment groups than in those treated with albendazole. Similarly, the time for 90% of the animals to reach target weight in the DQL-ABA treatments was 34 days ( $\pm 1$ ) shorter than for the albendazole treated animals.

Pasture sampling at the start of the experiment indicated no statistically significant difference in the number ( $P = 0.165$ ) or generic composition of the L3 populations on each farmlet ( $P > 0.4$  for both *T. circumcincta* and *Trichostrongylus* spp. and  $P = 0.121$  for *Nematodirus* spp.) (Table 2). Similarly, at the end of the experiment there was no difference between treatments in either the total number of L3 ( $P = 0.755$ ) or their generic composition ( $P = 0.147$ – $0.796$ ) (Table 2). There was, however, a

**Table 1**  
Mean values for production and parasite factors for each treatment with their associated  $P$  values.

Parameter	DQL-ABA	Albendazole	$P$ value
Mean faecal egg counts (epg)	199.4	660.0	0.001
Overall liveweight gain (kg)	25.43	16.43	0.001
Fleece weights (kg)	2.02	1.66	0.001
Carcass weight (kg)	23.36	18.64	0.001
Carcass value (NZ\$)	111.60	100.04	0.001
Fat depth (mm)	11.35	7.78	0.001
Condition score at 3rd treatment	2.7	2.3	0.026
Final condition score	3.41	2.44	0.001
Faecal soiling score at 3rd treatment	0.72	1.804	0.002
Final faecal soiling score	1.387	1.528	0.010



**Fig. 4.** Fitted logistic curves showing the progression of animals achieving the target weight of 38 kg when lambs were treated with DQL-ABA (—) or albendazole (---) over the course of the trial. The least significant difference (LSD) at the 5% level is given for the difference between the treatments.

**Table 2**

Mean parasite numbers and proportional genera mix on the pastures at the start and end of the experiment for both treatments. Tela – *Teladorsagia*, Trich – *Trichostrongylus*, Coop – *Cooperia*, Haem – *Haemonchus*, Oeso/Chab – *Oesophagostomum/Chabertia*, Nem – *Nematodirus*.

	Treatment	L3/kg dry matter	Tela	Trich	Coop	Haem	Oeso/Chab	Nem
Start	DQL-ABA	3357	18.7	7.0	0.0	0.0	0.0	73.3
	Albendazole	1690	24.0	13.4	0.0	0.0	0.0	62.5
End	DQL-ABA	88	49.3	4.4	9.4	6.5	0.0	13.6
	Albendazole	115	77.4	2.3	0.0	0.0	0.3	4.1

small population of *Haemonchus contortus* L3, which only occurred on three of the DQL+ABA farmlets with numbers <7 L3/kg dry matter ( $P=0.047$ ).

#### 4. Discussion

The production cost of using an anthelmintic which is not achieving the expected levels of efficacy due to anthelmintic resistance has been clearly demonstrated. Most notable in this study was the reduced liveweight gain of 9 kg over the trial period. This translated into a reduced carcass weight of 4.7 kg, which equated to a 10.4% reduction in carcass value. The effect of reduced parasite control was also seen in a range of other performance variables such as body condition, breech soiling and fleece weights.

The difference in liveweight gain measured in this trial was greater than observed in previous studies into the cost of anthelmintic resistance. Leathwick et al. (2008), Macchi et al. (2001) and Sutherland et al. (2010) recorded reductions of 3 kg, 1.3 kg and 2.8 kg in liveweight gains, respectively, from using anthelmintics which were compromised by the presence of resistance. These are all broadly similar but lower than the difference found in this experiment. This is likely due to a greater overall parasite challenge from L3 on the pastures at the start of this trial (Table 2), as indicated by the high FEC (Fig. 1) i.e. FECs in this study were generally higher throughout than those reported by Sutherland et al. (2010) and Macchi et al. (2001) which fluctuated around 100 and 500 epg, respectively.

A significant finding from this study was that, despite both groups of animals having relatively high FECs, all the lambs gained weight and appeared visually to be in good health. For example, the albendazole-treated lambs grew at an average rate of >110 g/day, and at the end of the study had an average body condition score of 2.44. This is important because many New Zealand farmers believe they will be able to detect an anthelmintic resistance problem in the performance of their stock. As a result, most farmers are reluctant to meet the cost of routine testing for anthelmintic resistance on their farms, and many have never done so (Lawrence et al., 2007). This lack of information regarding the resistance status of nematodes on their farm translates into a reluctance to implement resistance management practices. The results of this study demonstrate that even in the presence of established resistance in several nematode species, the losses accruing due to the presence of resistance can greatly exceed the cost of testing without any obvious visual signs of parasitism. Similarly, most New Zealand farmers are reluctant to make use of the two new classes of anthelmintic, now available for use in sheep in New Zealand (Mason et al., 2009; Little et al.,

2010), on the basis of price. However, the cost of using either of these new products, which can be relied upon to give high efficacy, is substantially less than the level of production loss shown in this study.

It was notable that over the course of the trial, L3 numbers on pasture declined, and there was no difference between treatments at the final comparison. This is likely to reflect the trial being run over the autumn-winter period, when development of nematode eggs passed onto pasture is at its lowest (Vlassoff et al., 2001). Thus, while the albendazole-treated animals would have deposited more nematode eggs onto pasture than the DQL-ABA-treated animals, this was not translated into a difference in L3 challenge to the grazing lambs because most of the eggs did not develop. Had the trial been run over the late spring to autumn period, it would reasonably have been expected that the use of a less than fully effective anthelmintic would have resulted in increased L3 numbers on pasture in the autumn. In a study conducted by Leathwick et al. (2008), increasing levels of infection resulting from failure of albendazole to control pasture contamination by resistant nematodes resulted in clinical parasitism and a premature conclusion to the study in order to avoid ethical issues. Had this aspect of using a less than fully effective anthelmintic been included in the current study it is likely that the observed productivity differences would have been even larger than presented here.

The timing of the trial is also the likely reason for the observed change in nematode genera present in the faecal cultures over the course of the trial (Fig. 2). As *T. circumcincta* is able to develop at colder temperatures than *Trichostrongylus* spp. (O'Connor et al., 2006), its eggs can continue to develop to L3 throughout the winter, albeit at a much reduced rate (Waghorn et al., 2011). This would be consistent with the observed increased proportion of *T. circumcincta* L3 on pasture in August (Table 2) and a greater proportion of this nematode in the infected lambs (Fig. 2). As sheep develop a strong immunity to *Nematodirus* spp. as they age, this species was largely absent from the faecal cultures by the end of the trial (Fig. 2), even though it was still represented in the L3 populations on pasture (Table 2).

Lambs in New Zealand are usually drafted for slaughter as they reach a specific target weight, optimally around 38 kg liveweight. The time required for animals to reach this target weight differed between treatments (Fig. 4). The opportunity to draft lambs to slaughter sooner would bring additional benefits to the farmer. Being able to sell animals sooner allows the farmer additional production, animal management and financial opportunities i.e. more pasture is available to feed the remaining animals, support the introduction of new stock or conserve for feed as hay



or silage. The value of these opportunities has not been estimated in the current study.

In conclusion, a loss in productivity due to subclinical parasitism has been clearly demonstrated by these results. Importantly, this loss, as seen in an earlier study (Sutherland et al., 2010), far exceeded the cost of routine testing of drench efficacy on a farm and/or the use of the new anthelmintic classes which are now available for use in New Zealand and other countries. There would appear to be a case for farmers to re-evaluate their stance on these issues as it is becoming clear that the potential losses caused by using an anthelmintic which is compromised by resistance can greatly exceed the cost of ensuring that effective treatments are used.

### Conflict of interest statement

This work was funded by Pfizer Australia Pty Ltd, West Ryde, NSW, Australia. There were no other potential conflicts of interest.

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